2857-Pos Board B549
Localized Nitric Oxide Signaling Mediates Cardiac Mechano-Chemotransduction
Zhong Jian, Huilan Han, Tieqiao Zhang, Jose Puglisi, Leighton T. Izu, John Shaw, Yi-Je Chen, Rafael Shimkunas, Nipavan Chiamvimonvat, Donald M. Bers, Kit S. Lam, **Ye Chen-Izu**.
UC Davis, Davis, CA, USA.
Cardiac myocytes contract against a mechanical afterload during each heartbeat. We have developed a novel Cell-in-Gel system to impose 3D mechanical stresses on single cardiac myocytes during contraction. Using this new technique, we identified key molecules involved in transducing mechanical stress to alter Ca^{2+} dynamics. Increasing mechanical load causes enhanced contractility and elevated systolic Ca^{2+} transient that is mediated by nitric oxide synthase (NOS). Increased load also causes a marked increase of diastolic spontaneous Ca^{2+} sparks, and their suppression is only effected by inhibition of nNOS but not eNOS. The differential effects on Ca^{2+} sparks may stem from the two-fold closer physical proximity of nNOS vs. eNOS to ryanodine receptors. In addition to NOS, NOX2 and CaMKII are also involved in the mechano-chemotransduction pathways, which together fine-tune cardiac contraction under mechanical load.

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Cardiomyopathy Ctnt Mutation in Patient Derived Cardiomyocytes from Induced Pluripotent Stem Cells Affects Sarcomere Structure and Function
Kathleen M. Broughton[1], Veronica Sanchez-Freire[2], Joseph C. Wu[2], Beata M. Wolska[3], Ross J. Solaro[3], Brenda Russell[3].
[1]Bioengineering, University of Illinois, Chicago, IL, USA, [2]Department of Medicine, Division of Cardiology, Stanford University, Stanford, CA, USA, [3]Physiology and Biophysics, University of Illinois, Chicago, IL, USA.
Cardiomyocytes derived from human induced pluripotent stem cells (hIPS-CM) are useful to understand basic structural and functional characteristics of normal and diseased human heart cells. We investigated mechanical properties of hIPS-CM derived from unaffected and affected members of a family harboring a dilated cardiomyopathy (DCM) mutation in cardiac troponin T (cTnT), the tropomyosin binding unit of troponin. Patients with a cTnT point mutation (R173W) develop DCM, which commonly leads to diastolic and systolic dysfunction and progressive heart failure. To study the shortening and shortening velocity of normal and cTnT mutant hIPS-CM cells, cells were plated on substrates with a relatively soft stiffness (160 kPa) molded from polydimethylsiloxane and measured using a line scan method. Data were collected using a Zeiss 710 confocal microscope. Visual observation of cells after 5 days maturity on the PDMS substrates indicate a smaller percent of mutant (8%) compared to normal (87%) hIPS-CM were spontaneously beating. Immunohistochemistry showed that myofibril structure was better developed in normal compared to cTnT mutation cells. Day 5 line scans revealed normal hIPS-CM cells shorten more (0.47 μm) than mutant cTnT cells (0.32 μm); shortening velocity was faster in normal (1.42 μm/s) compared to mutant cTnT cells (0.67 μm/s). Acute treatment (1 dose with a 10 min activation dwell time) with omecamtiv mecarbil (200nM), a cardiac myosin activator, increased the shortening and shortening velocity of normal (0.78 μm at 1.85 μm/s) but not cTnT mutant (0.32 μm at 0.70 μm/s) hIPS-CM cells. Although more studies are necessary, these results suggest that cTnT may affect development of sarcomeres and the regulation of contractility. Furthermore, the myosin activator omecamtiv mecarbil may not be sufficient to rescue dysfunction in the cTnT mutation R173W. [Funding: NIH T32HL07692, PO1HL62426]

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Contractile Structure-Function of Parvalbumin's Ef-Hand Metal Ion Binding Loop in Isolated Adult Cardiac Myocytes
Michelle L. Asp, Joseph M. Metzger.
Physiology, University of Minnesota, Minneapolis, MN, USA.
Parvalbumin (Parv), an EF-hand Ca^{2+} buffer, facilitates rapid relaxation in fast-twitch muscle. Parv gene delivery to the heart has been studied as a therapeutic strategy for diastolic heart failure, in which slow Ca^{2+} reuptake is an important contributor. A limitation of WT-Parv in this context is the significant trade-off between faster relaxation rate and blunting of contraction amplitude, which occurs because WT-Parv sequesters Ca^{2+} too early in the cardiac cycle and prematurely truncates sarcomere shortening in the facilitation of rapid relaxation. Our laboratory recently demonstrated that an E→Q substitution (ParvE101Q) at amino acid 12 of the EF-hand metal-ion binding loop increases Mg^{2+} affinity and decreases Ca^{2+} affinity. Mechanistically, E→Q disrupts bidentate Ca^{2+} binding at this site to reduce Ca^{2+} binding affinity. Functionally, this substitution delays Ca^{2+} buffering compared to WT-Parv in cardiac myocytes, which hastens relaxation without blunting contraction. Unexpectedly, ParvE101Q increases contraction amplitude above that of untreated myocytes and independent of Ca^{2+}, leading to the hypothesis that sarcomere-localized altered Mg^{2+} binding may contribute to the inotropic effect. In this work, to further elucidate the role of EF-hand motif residue 12 in function, ParvE101Q and ParvE101D-experessing cardiac myocytes are compared. Although both Q and D substitutions are thought to bind Ca^{2+} in a monodentate manner, ParvE101D may bind Mg^{2+} more strongly than ParvE101Q. Consistent with this, in preliminary studies, ParvE101D enhanced contraction amplitude to a greater extent than ParvE101Q. We will discuss these findings in the context of the mechanistic role of EF-hand loop residue 12 in conferring Ca^{2+} and Mg^{2+} binding affinities with the goal of optimizing a Ca^{2+} buffering system for heart failure.

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Phospholamban and Sarcolipin Pentamers Naturally Associate with the Sarcoplasmic Reticulum Calcium Pump
John Paul Glaves, Przemek Gorski, Joseph Primeau, Catharine Trieber, **Howard S. Young**.
Biochemistry, University of Alberta, Edmonton, AB, Canada.
Phospholamban and sarcolipin interact with the sarcoplasmic reticulum calcium pump (SERCA) and regulate contractility in smooth, cardiac and skeletal muscle. While both proteins can form oligomers, it is thought that only the monomers interact with and inhibit SERCA. To address the role of the phospholamban and sarcolipin pentamers, we have studied their interaction with SERCA using electron cryo-microscopy of two-dimensional co-crystals. In our previous studies, phospholamban oligomers were found interspersed between SERCA dimers and we constructed a three-dimensional model of the complex. We also addressed the molecular characteristics of phospholamban that contribute to its interaction with SERCA and we examined the effects of phosphorylation and mutation of phospholamban on the structure of the complex with SERCA. In our recent work, we compared two crystal forms of SERCA in the absence and presence of phospholamban by electron cryo-microscopy - namely, small helical crystals and large two-dimensional crystals. The SERCA dimer ribbons that are found in both crystal forms consist of a rigid assembly of calcium-free SERCA molecules. While the lattice formed by the SERCA dimer ribbons is different in the helical and two-dimensional crystals, we show that a phospholamban oligomer interacts with SERCA in a similar manner in both crystal types. With this information, we next undertook a structural investigation of SERCA and sarcolipin in the two-dimensional crystals. A projection map was determined for SERCA in the presence of sarcolipin to a resolution of 8.5 Å and was consistent with a pentameric state for sarcolipin. While both phospholamban and sarcolipin interacted with transmembrane segment M3 of SERCA, the interaction of the sarcolipin pentamer was mediated by an additional density consistent with a SLN monomer. We conclude that pentameric forms of both phospholamban and sarcolipin naturally associate with SERCA.

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Phospholamban C-Terminal Truncations Including Heart Failure Mutation L39Stop Decrease Membrane Localization and Oligomerization and Alter the Structure of the PLB-Serca Complex
Neha Abrol[1], Nikolai Smolin[1], Delaine K. Ceholski[2], Howard S. Young[2], Seth L. Robia[1].
[1]Cell and Molecular Physiology, Loyola University Chicago, Maywood, IL, USA, [2]Department of Biochemistry, University of Alberta, Edmonton, AB, Canada.
A naturally occurring missense Leu-39stop (L39X) mutation in phospholamban (PLB) results in truncation of the C-terminal transmembrane domain, leading to cardiomyopathy and premature death. In this study, we fused PLB and SERCA to fluorescent protein tags to determine the structural and thermodynamic consequences of progressive truncations of the C-terminal residues of PLB in the membranes of living cells. We found that deletion of only a few C-terminal residues resulted in significant loss of PLB membrane anchoring and mislocalization to the cytoplasm and nucleus. Selective permeabilization of the plasma membrane by saponin resulted in diffusion of fluorescently labeled PLB out of the cells, consistent with solubilization of truncated proteins. Western blot analysis showed the expected mobilities for truncation mutants relative to full length PLB-WT, indicating that the observed solubilization of PLB truncation mutants is not due to proteolysis. Moreover, molecular dynamics simulations recapitulated the observed loss of stable bilayer anchoring for truncated PLB. Fluorescence resonance energy transfer (FRET) analysis revealed that C-terminal truncations resulted in progressive loss of PLB-PLB FRET, indicating a decrease in the apparent affinity of PLB oligomerization. We quantified a similar decrease in the SERCA-PLB binding affinity. Despite this decrease in affinity, SERCA-PLB FRET was paradoxically increased by deletion of up to 4 C-terminal residues as a result of a 14 angstrom decrease